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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF PREVENTION
PESTICIDES AND
TOXIC SUBSTANCES

DATE: **August 2, 1999**

MEMORANDUM

SUBJECT: **BENOMYL AND CARBENDAZIM- RE-EVALUATION** - Report of the Hazard Identification Assessment Review Committee.

FROM: Deborah Smegal, Toxicologist Jan Route to DS
Re-Registration Branch 3
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chairman
And
Pauline Wagner, Co-Chairman Pauline Wagner 8/4/99
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Steve Knizner, Branch Senior Scientist
Re-Registration Branch 3
Health Effects Division (7509C)

PC Code: **Benomyl: 099101**
Carbendazim: 128872

BACKGROUND: On June 1, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) met to reassess the acute and chronic dietary, and dermal and inhalation endpoints for risk assessment for benomyl, and its primary metabolite carbendazim. The Committee's decisions are attached. This report supercedes the previous HIARC report dated 12/3/97 ; HED Doc No. 012418.

Committee Members in Attendance:

Dave Anderson, Bill Burnam, Virginia Dobozy, Pam Hurley, Mike Ioannou, Tina Levine, Sue Makris, Kathleen Raffaelle, Jess Rowland, P.V. Shah, and Brenda Tarplee (Executive Secretary).

Members in Absentia were Nicole Paquette and Pauline Wagner.

Other HED staff present at the meeting were Deborah Smegal, Re-Registration Branch 3.

Data Presentation: and Report Preparation

Deborah Smegal, MPH
Toxicologist

1. BACKGROUND

On January 9, 1997, the Health Effects Division's RfD/Peer Review Committee evaluated the toxicology data base of Benomyl and reassessed the Reference Dose but deferred to a later date the need for an additional Uncertainty Factor for the enhanced sensitivity to infants and children (as required by FQPA) as well as the final decision regarding the need for a developmental neurotoxicity study (Memorandum: G. Ghali, HED to C. Welch, RD, dated 05/28/97).

On January 14, 1997, the Health Effects Division's Toxicology Endpoint Selection Committee selected the doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments. but did not address the Margins of Exposure (MOEs) required for the various exposure scenarios.

On November 25, 1997, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) met to determine the Uncertainty Factors and the MOEs for dietary and non-dietary risk assessments as required by the Food Quality Protecting Act (FQPA) of 1996.

The HIARC Committee reconvened on June 1, 1999 to reassess the acute and chronic dietary RfDs as well as the dermal and inhalation endpoints for occupational and residential risk assessments for benomyl, and its primary metabolite of carbendazim or MBC. In foods and the environment, benomyl rapidly transforms to MBC. Hence, residues in food are primarily as MBC, and the EPA analytical method determines benomyl residues in food as MBC (i.e., the method involves hydrolysis, so any benomyl residue would be converted to MBC prior to analysis). Therefore, the HIARC selected doses and endpoints for risk assessment with MBC. At this meeting, the Committee also considered the Registrant's Rebuttal, Dated 4/22/98, and the Registrant proposed studies for use in endpoint selection. The Registrant believes that a number of conclusions reached in the RfD Peer Review Committee review of benomyl (01/09/97) and the report of the Hazard Identification Assessment Review Committee (12/03/97) were incorrect. HED has prepared detailed responses to each of the issues identified by the Registrant in memorandum from S. Makris and N. McCarroll to D. Smegal, June 30, 1999, D248200. This report supersedes the previous HIARC document (dated December 3, 1997, HED Doc No. 012418).

II. HAZARD IDENTIFICATION

A. BENOMYL

(1) **Acute Dietary Females 13+ (One-Day)**

Study Selected: Rat Developmental Studies for Benomyl

MRID. No. 00148393 (1980), 00115674, and 00126522 (1982)

Executive Summary:

In a developmental toxicity study conducted in Wistar rats (MRID 00148393), benomyl (99% was administered at dose levels of 0, 15.6, 21.2, 62.5, or 125 mg/kg/day body weight per day gestation days 7-16. The developmental NOAEL is 31.2 mg/kg/day and the LOAEL is 62.5 mg/kg/day based on increased fetal and litter incidence of ocular malformations (microophthalmia and anophthalmia), increased fetal mortality and significantly reduced fetal weight (percentages not provided). At the 125 mg/kg/day, there were increased fetal and litte incidences of malformation of the brain (distended lateral ventricles, hydrocephaly). This stu supports the findings of microophthalmia reported in the developmental study conducted in CRL:CD rats. Maternal toxicity was not present at the highest dose tested of 125 mg/kg/day.

In another developmental toxicity study in CHR:CD rats (MRID 00115674, and 00126522), benomyl (99.1%) was administered to pregnant rats on days 7-16 of gestation at gavage dose levels of 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg/day. The study was conducted to assess externa hydrocephaly and microophthalmia. Incidental observations of microophthalmia and hydrocephaly were observed at 62.5 mg/kg/day, which confirmed the LOAEL established in 1 1980 study. Based on the results, the NOAEL for microophthalmia is 30 mg/kg/day. Materna toxicity was not present at the highest dose tested of 62.5 mg/kg/day.

Dose and Endpoint for Establishing the acute RfD: Developmental NOAEL = 30 mg/kg/day based on increased incidence of microophthalmia at 62.5 mg/kg/day (LOAEL).

Comments about Studies and Endpoint: The developmental effects are presumed to occurr al a single dose (exposure).

Uncertainty Factor(s): 100 (10x for interspecies extrapolation and 10x for intraspecies variability).

Benomyl Acute RfD (Females 13+)= $\frac{30 \text{ mg/kg/day}}{100} =$ **0.3 mg/kg**

This risk assessment is required.

(2) Benomyl Acute Dietary (General Population) (One-Day)

Study Selected: Hess et al. 1991

MRID. No. Published literature

Executive Summary:

Adult male Sprague-Dawley rats (approximately 100 days of age, 20 rats/dose) were given a single gavage dose of 0, 25, 50, 100, 200, 400 or 800 mg/kg body weight benomyl (95% a.i.) i corn oil. Eight animals/group were sacrificed at 2 days and 12 animals/group at 70 days (exce for the 800 mg/kg group) after treatment. The testis and excurrent ducts were examined each time to determine benomyl effects on spermatogenesis and on the epididymis. The primary effects seen at day 2 were testicular swelling and occlusions of the efferent ductules. Prematu release of germ cells (sloughing) was the most sensitive short-term response to benomyl. At 2 and 50 mg/kg biologically significant sloughing occurred in 1% and 2.8% of the tubules, respectively. Sloughing was statistically significant ($p<0.05$) at doses of 100 mg/kg to 800 mg (approximately 25 to 55% of the tubules). Occlusions of the efferent ductules of the testis wer dose dependent at 0, 0, 10, 60, 83, 93 and 92% for the 0, 25, 50, 100, 200, 400 and 800 mg/kg groups, respectively and correlated with the increase in testis weight on day 2. Occluded effer ductules were identified by compacted luminal contents, swollen ductules and the presence of granulomas. Testes weight was significantly increased in the 200 to 800 mg/kg groups. Long term effects (70 days) were seen in the 100, 200 and 400 mg/kg groups, e.g., decreased testis weight (400 mg/kg), dose-dependent increases in seminiferous tubular atrophy, and increases i the number of reproductive tracts containing occluded efferent ductules. No long-term effects were seen in the 0, 25 or 50 mg/kg groups. The NOAEL is 25 mg/kg/day, and the LOAEL is mg/kg/day based on biologically significant sloughing and occlusions of the efferent ductules the testes.

Dose and Endpoint for Establishing the Acute RfD: NOAEL = 25 mg/kg/day for benomyl; LOAEL of 50 mg/kg/day, based on biologically significant premature release of germ cells (sloughing) in the testes, and occlusions of the efferent ductules of the testis 2 days postexpos

Comments about Study and Endpoint: This study identified lowest single dose associated wit testicular effects. All the single dose studies submitted by the registrant evaluated higher dose

Uncertainty Factor(s): 100 (10x for interspecies extrapolation and 10x for intraspecies variability).

Benomyl Acute RfD (Gen Pop)= $\frac{25 \text{ mg/kg/day}}{100}$ **= 0.25 mg/kg/day**

This risk assessment is required.

(3) Benomyl Chronic Dietary Risk Assessment (Reference Dose)

Study Selected: Two year Dog Study with Benomyl (Sherman et al. 1970)

MRID No. 00081913, 00097305

Executive Summary:

Groups of 4/sex/dose beagle dogs were administered a formulated product containing benomy the diet at dosage levels of 0, 100, 500 and 2500 ppm for 2 years. The dietary concentrations equivalent to 0, 2.5, 12.5 and 62.5 mg/kg/day ai benomyl. There were no treatment-related effects on mortality, hematology, urinalysis, or clinical signs Body weight gain and food consumption were decreased in the high dose group. Males in the high dose group had increa cholesterol, alkaline phosphatase and glutamic-pyruvic transaminase (GPT) values, as well as decreased total protein and albumin/globulin (A/G) ratio. Similar effects, other than cholester and total protein, were noted in the high dose females. The clinical chemistry observations support the adverse liver effects in the high dose group, characterized as cirrhosis and slight t marked bile duct proliferation in 4/6 dogs of the 2500 ppm (62.5 mg/kg/day ai) group. The NOAEL is 500 ppm (12.5 mg/kg/day ai) based on hepatic cirrhosis, clinical chemistry alterations as well as decreased weight gain and food consumption noted at 2500 ppm.

Dose/Endpoint for establishing the RfD: NOAEL = 12.5 mg/kg/day ai (500 ppm formulated product) based on a LOAEL of 62.5 mg/kg/day ai (2500 ppm formulated product) for hepatic cirrhosis, clinical chemistry alterations as well as decreased weight gain and food consumptio

Uncertainty Factor (UF): An uncertainty factor of 100 was applied to account for both interspecies extrapolation and intraspecies variability.

$$\textbf{Benomyl Chronic RfD} = \frac{\text{12.5 mg/kg/day (NOAEL)}}{\text{100 (UF)}} = \textbf{0.13 mg/kg/day}$$

This risk assessment is required.

(4) Benomyl Occupational/Residential Exposure Risk Assessments

(a). Benomyl Dermal Absorption

Study Selected: Rat dermal absorption study with benomyl (1979)

MRID No: 0097287

A dermal absorption study was conducted in rats (4 rats/time point/dose) using benomyl in the form of Benlate (50% WP). The test material was applied at dose levels of 0.2, 2, 20 or 200 m of Benlate (equivalent to 0.1, 1, 10 or 100 mg a.i. of benomyl). The exposure durations were (1, 2, 4 and 10 hours. The amount of benomyl absorbed ranged from 0.031 to 3.5 percent from the highest to the lowest dose, respectively, following the maximum exposure period of 10 ho This study is acceptable. No dermal absorption studies were located for MBC.

Dermal Absorption Factor: **3.5%**

(b). Benomyl Short- Term Dermal - (1 - 7 Days)

Study Selected: 21-day Dermal Toxicity Study in Rabbits with Benomyl

MRID. No. 00097287 (Hood et al. 1969)

Executive Summary:

In a 21-day dermal study conducted in New Zealand White Rabbits, benomyl (53% a.i.) was applied dermally to abraded dorsal skin for exposure durations of six hours a day for 5 days a week, for three weeks. The doses were equivalent to 0, 50, 250, 500, 1000 and 5000 mg/kg/d ai benomyl. There were 5 animals/sex/dose except for the high dose group where 2 animals/s were used. The test site was covered with a non-occlusive gauze pad, and all rabbits were fitt with plastic collars to prevent oral exposure. Moderate skin irritation was reported for all dos groups. At the two highest dose levels, systemic toxicity characterized by diarrhea, oliguria a hematuria were reported in females. In the 500 mg/kg/day dose group, males exhibited 19% a 20% decreases in testes weight and testes-to-body weight ratios, respectively. At 1000 mg/kg/day, males exhibited 30% and 24% decreases in testes weight and testes-to-body weig ratios, respectively (both non significant). This finding was not apparent at 5000 mg/kg/day, which may be attributed to the small number of animals evaluated at this dose (n=2 males). T were no treatment-related histopathological changes in the testes, except for one rabbit in the 5000 mg/kg/day group that had focal testicular degeneration. This study identifies a NOAEL LOAEL of 500 and 1000 mg/kg/day, respectively based on biologically significant decreased relative and absolute testes weights.

Dose and Endpoint for Risk Assessment: NOAEL = 500 mg/kg/day ai for benomyl based on decreases in relative and absolute testes weights at 1000 mg/kg/day ai (LOAEL).

Comments about Study and Endpoint: The testicular effects following dermal exposure are of concern since this was one of the target organ following oral exposure in other species (rats a dogs). The lack of effects at the highest dose tested (5000 mg/kg/day) may be due to the low number of animals (2/sex) evaluated at this dose.

This risk assessment is required.

(c). Benomyl Intermediate-Term Dermal - (7 days to Several Months)

Study Selected: 21-day Dermal Toxicity Study in Rabbits with Benomyl

MRID. No. 00097287 (Hood et al. 1969)

Executive Summary: See Short-Term

Dose and Endpoint for Risk Assessment: NOAEL = 500 mg/kg/day ai for benomyl based on decreases in relative and absolute testes weights at 1000 mg/kg/day ai (LOAEL).

Comments about Study and Endpoint: The testicular effects following dermal exposure are of concern since this was one of the target organs following oral exposure in other species (rats a dogs). The lack of effects at the highest dose tested (5000 mg/kg/day) may be due to the low number of animals (2/sex) evaluated at this dose.

This risk assessment is required.

(d). Benomyl Long-Term Dermal (Several months to lifetime)

Study Selected: Two year Dog Study with Benomyl (Sherman et al. 1970)

MRID No. 00081913, 00097305

Executive Summary: See Chronic Dietary for Benomyl

Dose and Endpoint for Risk Assessment: NOAEL= 500 ppm formulated product (12.5 mg/kg/day ai) for benomyl based on hepatic cirrhosis, clinical chemistry alterations as well as decreased weight gain and food consumption at 2500 ppm (62.5 mg/kg/day ai) (LOAEL). assessments.

Comments about Study and Endpoint: This dose was also used in establishing the benomyl R Since a dose from an oral study (i.e., oral NOAEL) was selected, a dermal absorption rate of 3.5% should be used for these risk

This risk assessment is required.

(e). Benomyl Inhalation Exposure (any time period; 1 Day to Several Months)

Study Selected: 90-day rat inhalation study with Benomyl (Warheit 1987)

MRID No(s). 40399501

Executive Summary

In a subchronic inhalation study, benomyl (95% a.i.) was administered to Crl:CDBR rats (Sprague Dawley) at concentrations of 0, 10, 50 and 200 mg/m^3 for 4 hours/day for 90 days. These concentrations are equivalent to doses of 0.96, 4.8, 19.2 mg/kg/day in males and 1.4, 7, and 28.8 mg/kg/day in females based on the average body weights of 300 and 220 grams for males and females, respectively in the study. The mass median aerodynamic diameters (MMA were in the range of 1.7 to 2.3 microns, with the smaller MMADs being reported at the lowest concentration, therefore, an adequate concentration of benomyl reached the lungs. Histological lesions suggestive of olfactory degeneration, characterized by necrosis, chronic and acute inflammation and loss of olfactory epithelium was observed at the highest dose tested in femal and at 50 mg/m^3 in males. At 200 mg/m^3, males also had decreased body weights (10.8%) and body weight gains (13.5%). The NOAEL is 10 mg/m^3 (0.96 mg/kg/day) for males. The LOAI is 50 mg/m^3 (4.8 mg/kg/day) based on olfactory degeneration in the nasal cavity.

Dose and Endpoint for Risk Assessment: NOAEL=10 mg/m^3 (0.96 mg/kg/day) based on olfactory degeneration in the nasal cavity at 50 mg/m^3 (4.8 mg/kg/day)(LOAEL).

Comments about Study and Endpoint: This dose/endpoint should be used for short, intermedia and long-term exposure risk assessments. HIARC recommends an additional uncertainty facto of 3 be applied to the NOAEL (i.e., MOE = 300).

This risk assessment is required.

B. CARBENDAZIM (MBC)

(1) MBC Acute Dietary Females 13+ (One-Day)

Study Selected: Rat Developmental Study for Carbendazim (MBC)

MRID. No. 40438001 (Alvarez 1987)

Executive Summary:

In a developmental toxicity study (MRID No.: 40438001), 25 Crl:CE BR strain presumed pregnant rats per dose group were dosed with 0, 5, 10, 20 or 90 mg/kg/day of carbendazim (MBC, 98.8% a.i. in 0.5% methyl cellulose) by gavage on days 7 through 16 of gestation. Th rats were sacrificed on day 22 of gestation. There was no effect on maternal body weight or other extrauterine parameters but there was a 10% increase ($p < 0.05$) in absolute liver weight : 90 mg/kg/day. Lower mean body weight on days 17-22 of gestation in the high dose group pri to sacrifice was attributed to intrauterine effects such as resorptions and not maternal toxicity. There were 24, 23, 24, 22 and 15 dams that delivered pups for the control to high dose groups, respectively. The low number of dams delivering pups in the high dose group was attributed t only 19 dams being pregnant in this group as well as one death (by mechanical dosing trauma) and three dams with total resorptions. **The LOAEL is 90 mg/kg/day based on absolute live weight increase. The NOAEL is 20 mg/kg/day.**

There were a total of 312, 310, 281, 288 and 149 fetuses for the control to high dose groups, respectively available for examination. At 20 mg/kg/day there was a decrease in **fetal weight** (-5% $p < 0.05$ for combined sexes). The mean percent of fetuses with **variations due to retarded development** was 22.9%, 25.0%, 19.5%, 41.6% ($p < 0.5$) and 52.5% ($p < 0.05$) for t control to high dose groups, respectively. At 20 mg/kg/day the vertebrae showed increases in bipartite ossification (21 incidents in 8 litters) and dumbbelled centrum (44 incidents in 13 litters) due to retarded development as well as misaligned sternebrae and extra ossification of t ribs that were not described as being related to retarded growth. At 90 mg/kg/day, there were : variety of developmental effects including decreases in mean live fetuses per litter (-24%, $p<0.05$), early and late resorptions, and decreased fetal weight (-26%, $p < 0.05$, both sexes combined). There were 3/2, 1/1, 1/1, 3/3, and 91/15 ($p < 0.01$) fetuses/litters affected with **malformations** in the control to high dose groups, respectively. These malformations include variety of conditions mainly in the head (exencephaly, domed head), eyes (none, small or bul paws (clubbed) and skeleton (fused vertebrae, ribs and sternum or malformed scapula). Som these malformations were seen in the three fetuses affected in the 20 mg/kg/day dose group an not in the lower doses or controls. Thus, 20 mg/kg/day is considered a threshold for malformations. **The LOAEL is 20 mg/kg/day based on decreased fetal body weight and increases in skeletal variations and a threshold for malformations. The NOAEL is 10 mg/kg/day.**

Dose and Endpoint for Establishing the acute RfD: Developmental NOAEL = 10 mg/kg/day fc MBC based on decreased fetal body weight and increases in skeletal variations and a threshold for malformations in dams exposed to 20 mg/kg/day (LOAEL). MBC is the primary metabolit of benomyl.

Comments about Study and Endpoint: HIARC concluded that the NOAEL is appropriate for t exposure period (i.e., after a single dose). The acute RfD is based on MBC, the primary metabolite of benomyl. The developmental NOAEL of 10 mg/kg/day for MBC is lower than t developmental NOAEL of 30 mg/kg/day for benomyl. Fetuses of pregnant dams exposed to 62.5 mg/kg/day benomyl during gestation days 7 through 16 had an increased incidence of microophthalmia. In the environment, benomyl rapidly transforms to MBC. Hence, residues food are primarily as MBC, and the EPA analytical method developed to determine benomyl residues in food only measures MBC (i.e., the method involves hydrolysis, so any benomyl residue would be converted to MBC prior to analysis).

Uncertainty Factor(s): 100 (10x for interspecies extrapolation and 10x for intraspecies variability).

$$\textbf{MBC Acute RfD (Females 13+)} = \frac{10 \text{ mg/kg/day}}{100} = \textbf{0.1 mg/kg}$$

This risk assessment is required.

(2) MBC Acute Dietary (General Population) (One-Day)

Study Selected: Nakai et al. 1992

MRID. No. Published literature

Executive Summary:

Groups of male rats (n=20/dose between 97 and 105 days of age) were treated with a single or dose of 0, 50, 100, 200, 400 or 800 mg/kg MBC and killed on day 2 or 70 post-treatment. On day 2, at 50 mg/kg, round spermatids were sloughed (prematurely released) from stage I and I epithelium and elongated spermatids were sloughed from stage VII epithelium. In addition a dose-dependent increase in testicular weight was seen at dose levels of 100 mg/kg and higher that was accompanied by significant increases in mean seminiferous tubular diameter at 400 a 800 mg/kg. At 100 mg/kg, the disappearance of germ cells was more severe and statistically significant and sloughing of elongated spermatids extended into stages XII and XIV. In anim treated with 100 mg/kg or more, there was a dose-dependent increased incidence of occlusion the efferent ductules of the testes. The rete testis was swollen with sloughed germ cells indicating that ductal blockage had occurred further down the tract. At doses of 200 mg/kg ar above, missing germ cells extended into all stages except stages IX-XI, while, at doses of 400 800 mg/kg, some of the seminiferous epithelia were damaged so severely that it was difficult identify the stage.

On day 70, tubule diameter was significantly decreased at all doses in a dose-dependent relationship. Histologically, these decreases were associated with a dose-dependent increase ir seminiferous tubular atrophy (significant at 100 mg/kg and higher). No atrophic tubules were seen in the control rats, however, atrophy of a few seminiferous tubules in one testicle was not at 50 mg/kg. The atrophied tubules contained primarily Sertoli cells and occasional spermatogonia and were surrounded by a thickened basement membrane. Pathological alterations were also noted in the efferent ductules of the treated animals, 50% or more of the ducts being occluded in rats dosed with 100 mg/kg or more. Minimal effects were seen at 50 mg/kg, where slight abnormal growth of the efferent ductules was seen in only one specimen. The occlusions were characterized as compacted luminal contents, spermatic granulomas, mineralizations and obliterations of the original lumen by fibrotic connective tissue. In additic mean testis weight showed a dose-dependent decrease that was statistically significant at dose: 100 mg/kg and greater.

Dose and Endpoint for Establishing the acute RfD: LOAEL = 50 mg/kg/day for MBC; no NOAEL was identified based on sloughing (premature release) of immature germ cells 2 days postexposure; and atrophy of a few seminiferous tubules in one testicle, significant decrease ir seminiferous tubule diameter, and slight abnormal growth of the efferent ductules at 70 days postexposure. The subtle effects detected in the epididymal sperm at 50 mg/kg may be attribu to the direct effect of MBC on the seminiferous epithelium.

Comments about Study and Endpoint: The testicular effects were seen 2 days post exposure ai this study identified lowest single dose associated with testicular effects. All the single dose studies submitted by the registrant evaluated higher doses.

Uncertainty Factor(s): 100 (10x for interspecies extrapolation and 10x for intraspecies variabil 3x for lack of a NOAEL).

MBC Acute RfD (Gen Pop)= $\frac{25 \text{ mg/kg/day}}{300} =$ **0.17 mg/kg/day**

This risk assessment is required.

(1) **MBC Chronic Dietary Risk Assessment (Reference Dose)**

The chronic MBC RfD established in December 3, 1997 by the HIARC was re-assessed and t NOAELs and LOAELs were reaffirmed by the HIARC in pursuant of the FQPA.

Study Selected: Chronic toxicity study in Dogs with MBC (Sherman et al. 1972)

MRID No. 00088333

Executive Summary:

Beagle dogs (4/sex/dose) were administered a product formulation containing 53% a.i. carbendazim, a primary metabolite of benomyl at dietary doses levels of 0, 100, 500 or 2500 pp for two years. This is equivalent to 0, 2.5, 12.5 or 62.5 mg/kg/day ai MBC. No treatment-relat effects were noted in dogs fed 100 ppm (2.5 ai mg/kg/day). Dogs of both sexes in the mid dos group (500 ppm or 12.5 ai mg/kg/day) exhibited liver pathology characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis. At 500 ppm, there were also reported increases in cholesterol, total protein, SGPT and alkaline phosphatase, none of which were biologically or statistically significant. At 2500 ppm (62.5 mg/kg/day ai), anorexia, distended abdomens and poor nutritional condition were reported.

The NOAEL is 100 ppm (2.5 mg/kg/day ai). The LOAEL is 500 ppm (12.5 mg/kg/day ai) bas on histopathological lesions of the liver characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis in both sexes of dogs.

Dose/Endpoint for establishing the RfD: NOAEL = 2.5 mg/kg/day ai for MBC based on histopathological lesions of the liver characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis in both sexes of dogs at 12.5 mg/kg/day ai (LOAEL).

Comments about Study and Endpoint: The chronic RfD is based on MBC, the primary metabolite of benomyl. In foods and the environment, benomyl rapidly transforms to MBC. Hence, residues in food are primarily as MBC, and the EPA analytical method determines benomyl residues in food as MBC (i.e., the method involves hydrolysis, so any benomyl resid would be converted to MBC prior to analysis).

Uncertainty Factor (UF): An uncertainty factor of 100 was applied to account for both interspecies extrapolation and intraspecies variability.

$$\textbf{MBC Chronic RfD} = \frac{\text{2.5 mg/kg/day (NOAEL)}}{\text{100 (UF)}} = \textbf{0.025 mg/kg/day}$$

This risk assessment is required.

(2) MBC Occupational/Residential Exposure Risk Assessments

(a). Dermal Absorption

No dermal absorption studies are available for MBC, therefore, the dermal absorption factor o 3.5% for benomyl should be used.

(b). **MBC Short-Term Dermal - (1 - 7 days)**

Study Selected: Rat Developmental Study for MBC

MRID. No. 40438001 (Alvarez 1987)

Executive Summary: See MBC Acute Dietary for Females 13+

Dose and Endpoint for Risk Assessment: Developmental NOAEL = 10 mg/kg/day for MBC based on decreased fetal body weight and increases in skeletal variations and a threshold for malformations in dams exposed to 20 mg/kg/day (LOAEL). MBC is the primary metabolite of benomyl. Since a dose from an oral study (i.e., oral NOAEL) was selected, a dermal absorption rate of 3.5% should be used for these risk assessments.

Comments about Study and Endpoint: No dermal toxicity studies were located for MBC. The oral (developmental) NOAEL was selected because of the concern for developmental effects seen with MBC as well as the parent compound Benomyl. The HIARC requests that the registrant submit a 21-day dermal toxicity study in rats with MBC.

In the environment, benomyl rapidly transforms to MBC. Hence, workers and residents are likely to be exposed to MBC following occupational and residential uses of MBC.

Since an oral value was selected, 3% dermal absorption factor should be used in risk assessments.

This risk assessment is required.

(c). **MBC Intemediate -Term Dermal - (7 days to Several Months)**

Study Selected: Rat Developmental Study for MBC

MRID. No. 40438001 (Alvarez 1987)

Executive Summary: See MBC Acute Dietary for Females 13+

Dose and Endpoint for Risk Assessment: Developmental NOAEL = 10 mg/kg/day for MBC based on decreased fetal body weight and increases in skeletal variations and a threshold for malformations in dams exposed to 20 mg/kg/day (LOAEL). MBC is the primary metabolite o benomyl. Since a dose from an oral study (i.e., oral NOAEL) was selected, a dermal absorptio rate of 3.5% should be used for these risk assessments.

Comments about Study and Endpoint: No dermal toxicity studies were located for MBC. The oral (developmental) NOAEL was selected because of the concern for developmental effects seen with MBC as well as the parent compound Benomyl. The HIARC requests that the registrant submit a 21-day dermal toxicity study in rats with MBC.

In the environment, benomyl rapidly transforms to MBC. Hence, workers and residents are likely to be exposed to MBC following occupational and residential uses of MBC.

Since an oral value was selected, 3% dermal absorption factor should be used in risk assessments.

(d). MBC Long-Term Dermal (Several months to lifetime)

Study Selected: Chronic toxicity study in Dogs with MBC (Sherman et al. 1972)

MRID No. 00088333

Executive Summary: See Chronic Dietary for MBC

Dose and Endpoint for Risk Assessment: NOAEL= 100 ppm (2.5 mg/kg/day ai) for MBC bas on histopathological lesions of the liver characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis in both sexes at 500 ppm (12.5 mg/kg/day) (LOAEL). .

Comments about Study and Endpoint: This dose/endpoint was also used in establishing the M RfD. Since a dose from an oral study (i.e., oral NOAEL) was selected, a dermal absorption ra of 3.5% should be used for these risk assessments.

This risk assessment is required.

(e). MBC Inhalation Exposure (any time period; 1 Day to Several Months)

Study Selected: 90-day rat inhalation study with Benomyl (Warheit 1987)

MRID No(s). 40399501

Executive Summary See Benomyl Inhalation Exposure

Dose and Endpoint for Risk Assessment: NOAEL= 0.96 mg/kg/day based on olfactory degeneration in the nasal cavity. This dose is lower than the developmental NOAEL for MBC 10 mg/kg/day, and therefore would be protective of the *in utero* developmental effects of concern.

Comments about Study and Endpoint: There are no inhalation studies available for MBC, therefore, the benomyl inhalation study is used to assess MBC inhalation exposures. For long-term exposures (> several months), HIARC recommends an additional uncertainty factor of 3 b applied to the NOAEL (i.e., MOE = 300).

This risk assessment is required.

C. **Margins of Exposure for Occupational/Residential Exposures:**

A MOE of 100 should be used for all of the occupational risk assessment scenarios with benon and MBC because these endpoints were based on NOAELs from animal studies, except for the long-term inhalation exposures, where a MOE of 300 should be used. An additional uncertaint factor of 3 was applied to the subchronic inhalation study to be protective of longer-term exposure more than several months. The MOEs for residential exposure risk assessment scenarios will be determined by the FQPA Safety Factor Committee.

D. **Recommendations for Aggregate Exposure Risk Assessments**

For both benomyl and MBC **acute** aggregate exposure risk assessments, combine the high end exposure values from food + water for the population of concern (i.e., females 13+, or the general population) and compare it to the appropriate acute RfD.

Short- or Intermediate-Term aggregate exposure risk assessment is not appropriate for benomyl or MBC because the endpoints are different for dermal (testicular effects for benomy and developmental for MBC), inhalation (respiratory effects), and oral (liver effects) exposure

For **Long-Term** aggregate exposure risk assessment, the oral and dermal exposures, which are both based on liver effects, should be aggregated. The dermal exposure should be converted t an oral equivalent dose (using 3.5% dermal absorption) and compared to the oral NOAEL. Inhalation effects are not aggregated because the endpoint is based on respiratory tract effects.

III. FQPA CONSIDERATIONS

1. Neurotoxicity Data

This issue was previously addressed in the December 3, 1997 HIARC Report (HED Document No. 012418), and is discussed below under Determination of Susceptibility and Recommendation for a Developmental Neurotoxicity Study.

2. Determination of Susceptibility.

For this risk assessment. HIARC determined that the **10 x** factor to account for enhanced sensitivity of infants and children **(as required by FQPA) should be retained.** Although no increased sensitivity was observed for benomyl in young rabbits following *in utero* exposure or in pups as compared to adu in the two-generation reproduction study in rats, **HIARC recommends that the FQPA 10x for benomyl and MBC be retained** because:

(i) There is increased sensitivity of rat fetuses as compared to maternal animals following *in utero* exposure in a prenatal developmental toxicity study in rats fc benomyl. Increased sensitivity manifested as developmental anomalies (decreased fetal body weight and ocular and/or cerebral malformations) at dose: which were found to be not maternally toxic. For developmental toxicity the NOAEL was 30 mg/kg/day whereas for maternal toxicity, the NOAEL was ≥12 mg/kg/day (highest dose tested).

(ii) There is concern for the developmental neurotoxic potential of Benomyl.

a) In a pre-natal developmental toxicity study conducted under Subdivision F Guidelines, malformations of the CNS (e.g., anophthalmia, microophthalmia, a hydrocephaly) were observed in rat fetuses following prenatal exposure to benomyl.

b) In addition, there is extensive evidence from the published literature which indicates that benomyl produces CNS anomalies in rats when administered duri gestation, including some studies which suggest that this effect is enhanced by dosing in late gestation.

c) These concerns are also supported by the evidence of neurotoxic effects in t acute and subchronic neurotoxicity (Subdivision F Guideline) studies

d) Developmental neurotoxicity studies with benomyl and MBC are required. The absence of these studies results in uncertainties regarding the evaluation of hazards to infants and children.

(iii) There is increased sensitivity of rat and rabbit fetuses as compared to maternal animals following *in utero* exposure to MBC, the primary metabolite of benom in prenatal developmental toxicity studies. In the MBC rat study, increased sensitivity manifested as developmental anomalies (decreased fetal body weigh and increases in skeletal variations and a threshold for malformations) at doses which were not maternally toxic. For developmental toxicity the NOAEL was mg/kg/day, whereas for maternal toxicity, the NOAEL was 20 mg/kg/day (base on a slight increase in liver weight at 90 mg/kg/day).

In the rabbit developmental study with MBC, increased sensitivity manifested a decreased implantations and litter size, and increased resorptions at 20 mg/kg/d the NOAEL is 10 mg/kg/day. Maternal toxicity was not observed until higher doses of 125 mg/kg/day, based on abortions and decreased maternal body weig the maternal NOAEL is 20 mg/kg/day.

(iii) Mutagenicity studies with benomyl and MBC provide evidence of aneuploidy induction following oral dosing in mice. The mutagenicity data support the evidence of developmental anomalies in rats.

3. Recommendation for a Developmental Neurotoxicity Study

BENOMYL

The previous decision by HIARC on December 3, 1997 to require a developmental neurotoxicity stud for benomyl was re-assessed based on the registrant's rebuttal Dated April 22, 1998, and re-affirmed b HIARC on June 1, 1999. The following weight-of-the-evidence was considered by the committee:

- The prenatal developmental toxicity study in rats with benomyl demonstrated central nervous system (CNS) anomalies in the fetuses following maternal exposure during gestation. The CNS anomalies included anophthalmia, microophthalmia, and hydrocephaly (MRID No. 00148393 and 0015764).

- A number of other studies on benomyl available in the literature have also demonstrated similar observations (Zeman et al., 1986; Ellis et al., 1987, 1988; Hess et al., 1987; Hoogenboom et al., 1991 and Lu et al., 1994).

- There is a suggestion that administration of Benomyl in late gestation, as oppos to administration only during the period of major organogenesis, enhances the incidences of CNS anomalies in rats (Zeman et al., 1987 and Ellis et al., 1988).

- In mutagenicity studies with benomyl, there is evidence of aneuploidy inductio following oral dosing in mice (MRID No. 42911601, 42911602). Mutagenicit data support the evidence of developmental anomalies in rats. Hoogenboom et (1991) postulated that the known antitubulin action of Benomyl may impair microtubule formation and produce brain and ocular malformations by disrupti of neuronal proliferation and migration.

- In an acute neurotoxicity study a single dose of Benomyl at 2000 mg/kg causec decrease in motor activity in females along with a decrease in body weight gai Therefore, the former effect was not considered to be evidence of neurotoxicity

On the other hand, the decrease (6%) in absolute brain weight in males at 500 (2000 mg/kg was considered to be a possible indicator of neurotoxicity (MRID 42817003).

- In a subchronic neurotoxicity study in rats, the increased motor activity observ in females given repeated oral administration of Benomyl at 578 mg/kg/day w considered to be indicative of a possible neurotoxic effect in light of FQPA. T Committee noted that functional effects were not measured in this study (MRI No. 43277901).

MBC

The HIARC determined that a developmental neurotoxicity study *is required* for MBC. The followi weight-of-the-evidence was consid:red by the committee:

- Developmental CNS malformations. In a prenatal developmental toxicity stu rats with MBC, treatment-related malformations of the CNS were observed. These included exencephaly, domed head, anophthalmia, microophthalmia an bulged eyes.

- There is increased sensitivity of rat and rabbit fetuses as compared to materna animals following *in utero* exposure to MBC, the primary metabolite of benor in prenatal developmental toxicity studies.

- In mutagenicity studies with MBC, there is evidence of aneuploidy induction following oral dosing in mice (MRID No. 42911602). Mutagenicity data sup the evidence of developmental anomalies in rats.

The Committee recommended that highest dose level tested in the developmental neurotoxicity stud should be sufficiently high to demonstrate the CNS defects observed in other studies. A clear differential in fetal response to gavage versus dietary exposure to Benomyl has been demonstrated, gavage dosing producing anomalies at approximately one-tenth of the dietary level (Kavlock et al., 1982; Chernoff, 1985). This would need to be considered when the protocol is designed and dose le are selected for the developmental neurotoxicity study. Due to greater exposure concerns for MBC, HIARC would prefer that the registrant give higher priority to conducting the developmental neurotoxicity study for MBC.

IV. RECOMMENDATION FOR ADDITIONAL STUDIES

The HIARC recommended that the following additional studies be conducted: (1) 870.3200 21-day dermal toxicity study in rats with MBC; (2) 870.6300- Developmental neurotoxicity study in the ra with benomyl; and (3) 870.6300- Developmental neurotoxicity study in the rat with MBC. The rationale for requiring developmental neurotoxicity studies for benomyl and MBC is provided in Se III under "Recommendations for a Developmental Neurotoxicity Study."

V. ACUTE TOXICITY

Acute Toxicity of Benomyl					
Guideline No.	Study Type	% a.i.	MRID #	Results	Toxicity Category
81-1	Acute Oral, Rat	75	00064819	LD_{50} = >5000 mg/kg,	IV
81-2	Acute Dermal, Rat	75	243043	LD_{50} = >2000 mg/kg,	III
81-3	Acute Inhalation, Rat	50	00097599	LC_{50} >4.01 mg/L	III
81-4	Primary Eye Irritation, Rabbit	75	00064820	irritant	II
81-5	Primary Skin Irritation, Rabbit	75	243043	Non-irritant	IV
81-6	Dermal Sensitization, Guinea Pig	not given	050427	mild to moderate dermal sensitizer	N/A
81-7	Delayed neurotoxicity, hen	not given	241930	NOAEL = 2500 mg/kg	N/A
81-8	Acute Neurotoxicity, Rat	97.4	42817003	NOAEL >2000 mg/kg	N/A

N/A Not applicable

VI. SUMMARY OF TOXICOLOGY ENDPOINTS SELECTION: BENOMYL

The doses and toxicological endpoints selected for various exposure scenarios are summarized below:

Summary of RfDs and Toxicological Endpoints for Benomyl			
EXPOSURE SCENARIO	**DOSE (mg/kg/day)**	**ENDPOINT**	**STUDY**
Acute Dietary, Females 13+	NOAEL=30 UF = 100	Increased incidence of microophthalmia at 62.5 mg/kg/day (LOAEL) in pregnant rats given oral administrations of Benomyl at during gestation days 7 through 16.	Rat Developmental Study with Benomyl
Acute Dietary, General Population	NOAEL=25 UF =100	Biologically significant premature release of germ cells (sloughing) in the testes, and occlusions of the efferent ductules of the testis 2 days postexposure	Single Dose Rat Study (Hess et al. 1991)
Benomyl Acute RfD (Females 13+) = 0.3 mg/kg/day **Benomyl Acute RfD (General Population) = 0.25 mg/kg/day**			
Chronic Dietary	NOAEL= 12.5 UF= 100	Hepatic cirrhosis, clinical chemistry alterations as well as decreased weight gain and food consumption.	2 year dog study with benomyl
	Benomyl Chronic RfD =0.13 mg/kg/day		
Short-and Intermediate Term Dermal	Dermal NOAEL = 500	Decreases in relative and absolute testes weights	21 Day Dermal Rabbit Study
Long-Term Dermal [a]	Oral NOAEL =12.5	Hepatic cirrhosis, clinical chemistry alterations as well as decreased weight gain and food consumption	2 year dog study with benomyl
Short-, Intermediate- and Long Term Inhalation	Inhalation NOAEL= 0.96 (10 mg/m^3)	Olfactory degeneration in the nasal cavity	90 day rat inhalation study

a = Since an oral value was selected, 3.5% dermal absorption factor should be used for route-to-route extrapolation.

VII. SUMMARY OF TOXICOLOGY ENDPOINTS SELECTION: CARBENDAZIM

The doses and toxicological endpoints selected for various exposure scenarios are summarized below:

Summary of RfDs and Toxicological Endpoints for CARBENDAZIM (MBC)			
EXPOSURE SCENARIO	**DOSE (mg/kg/day)**	**ENDPOINT**	**STUDY**
Acute Dietary, Females 13+	NOAEL=10 UF = 100	Decreased fetal body weight and increases in skeletal variations and a threshold for malformations	Rat Developmental Study with MBC
Acute Dietary, General Population	LOAEL=50 UF = 300	Sloughing (premature release) of immature germ cells 2 days postexposure, atrophy of a few seminiferous tubules in one testicle, significant decrease in seminiferous tubule diameter, and slight abnormal growth of the efferent ductules at 70 days postexposure.	Single Dose Rat Study (Nakai et al. 1992)
MBC Acute RfD(Females 13+) =0.1 mg/kg/day MBC Acute RfD(General Population) =0.17 mg/kg/day			
Chronic Dietary	NOAEL= 2.5 UF= 100	Histopathological lesions of the liver characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis in both sexes of	2 year dog study with MBC
	MBC Chronic RfD =0.025 mg/kg/day		
Short-and Intermediate Term Dermal [a]	Oral NOAEL =10	Decreased fetal body weight and increases in skeletal variations and a threshold for malformations in dams	Rat Developmental Study with MBC
Long-Term Dermal [a]	Oral NOAEL =2.5	Histopathological lesions of the liver characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis in both sexes of dogs	2 year dog study with MBC
Short-, Intermediate- and Long Term Inhalation	Inhalation NOAEL= 0.96 (10 mg/m^3)	Olfactory degeneration in the nasal cavity	90 day rat inhalation study

a = Since an oral value was selected, 3.5% dermal absorption factor should be used for route-to-route extrapolation.

VII. BIBLIOGRAPHY

Chernoff, N. (1985). The Science of Teratology in a Regulatory Setting. *Basic Life Science* 34:285-30

Ellis, WG, Semple, JL, Hoogenbbom, ER, Kavlock, RJ and Zeman. FJ. (1987). Benomyl-Induced Craniocerebral Anomalies in Fetuses of Adequately Nourished and Protein-Deprived Rats. *Teratogenesis, Carcinogenesis and Mutagenesis* 7(4):357-375.

Ellis, WG, DeRoss, F, Kavlock, RJ and Zeman, FJ. (1988). Relationship of Periventricular Overgrowt to Hydrocephalus in Brains of Fetal Rats Exposed to Benomyl. T*eratogenesis, Carcinogenesis and Mutagenesis* 8(6):377-392.

Hess, N, Ellis, W, Kavlock, RJ and Zeman, F. (1987). Fetal Brain Development in the Malnourished Benomyl-Exposed Rat. *Federal Proceedings* 4693):956.

Hess, RA, Moore, BJ, Forrer, J., Linder, RE and Abuel-Atta. 1991. The fungicide benomyl (methyl) (butylcarbomyl)-2-benzimidazole carbamate causes testicular dysfunction by inducing the sloughing c germ cells and occlusion of efferent ductules. Fundam. Appl. Toxicol. 17:733-745

Hoogenboom, ER, Ransdell, JF, Ellis, WG, Kavlock, RJ and Zeman, FJ. (1991). Effects on the Fetal Rat Eye of Maternal Benomyl Exposure and Protein Malnutrition. *Current Eye Research* 10(7):601-612.

Kavlock, RJ, Chernoff, N, Gray, LE, Gray, JA and Whitehouse, D. (1982). Teratogenic Effects of Benomyl in the Wistar Rat and CD-1 Mouse, with Emphasis on the Route of Administration. *Toxicol.Appl.Pharmacol.* 62(1):44-54.

Lu, SY, Lin, HW and Wang, SC. (1994). Studies on Teratogenicity of Benomyl in Rats. *J. Chinese S Vet. Science* 20(4):348-356.

Nakai, M., Hess, RA, Moore, BJ, Guttroff, RF, Strader LF, and Linder RE. 1992.-Acute and long-ter effects of a single dose of the fungicide carbendazim (methyl 2-benzimidazole carbamate) on the male reproductive system in the rat. J. Androl. 13:507-518.

Zeman, FJ, Hoogenboom, ER, Kavlock, RJ and Semple, JL. (1986). Effects of the Fetus of Maternal Benomyl in the Protein-Deprived Rat. *J. Toxicol. Environ. Health* 17(4):405-417.